**Sewage-based epidemiology to track down the use of new psychoactive substances: challenges and pitfalls**

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Due to their unknown effects and often harmful side effects, New Psychoactive Substances (NPS) may present public health consequences, while adding new challenges to law enforcement. These substances have similar effects to classical illicit drugs (e.g. cannabis, ecstasy or amphetamine), are often structurally related with them, and are specifically designed to circumvent existing legislation. NPS include, among other, synthetic cannabinoids, synthetic cathinones, phenylethylamines, piperazines, and tryptamines. To assist in the control of NPS, early warning systems and policy makers require information on their trends, usage patterns and prevalence. The current system of detecting and restricting NPS is no longer fit for purpose, and the European Union has declared the NPS issue as a priority, from research and policy perspectives. In most cases, no information is available regarding effects, toxicity, metabolic aspects, abuse liability, and interactions with other (prescription) drugs and/or alcohol.

There are several (complementary) approaches which can be used to gather information on the use of NPS, each with its own challenges and pitfalls.

1) Identification of NPS in suspicious drug preparations, such as tablets, powders, capsules and herbal blends, collected from amnesty bins (placed at the entrance of festivals and dance events), purchased from online smart shops and head shops, and from police seizures.

2) Identification of NPS from clinical intoxication cases. While such approach might provide information on the current use of NPS, it sometimes encounters difficulties in interpretation due to the lack of knowledge related to human metabolism of NPS. *In vitro* approaches for quick metabolism may be needed for correct data interpretation.

3) Identification of NPS in pooled urine collected from the reservoirs of portable stand-alone urinals placed in strategic locations, such as festivals, dance events, nightlife areas. Pooled urine analysis is representative for specific populations (recreational settings) and is regarded as an unconventional and innovative approach to deliver objective and timely information on NPS use. However, this purely qualitative approach might be biased towards the NPS use by men and might suffer from “dilution” of the (few) positive samples with a larger number of negative urine samples.

4) Analysis of NPS in sewage samples (collected at the inlet of sewage treatment plants) aims to further add to the wealth of information and to complement the current methods of detection. The sewage results provide real time estimates of community NPS use, and can generate spatial trends at local and national levels in a timely and relatively cheap manner. Yet, sewage-based epidemiology relies on a sufficient number of users so that the concentration of NPS (or their metabolites) in sewage is detectable.

The identification and quantification of NPS and their metabolites (biomarkers) in complex matrices, such as pooled urine and sewage, requires advanced analytical techniques, such as chromatography coupled to (high resolution) mass spectrometry because of their high selectivity and sensitivity of measurements. These approaches might be tedious since NPS are not included in the available (commercial) mass spectral databases and the identification and confirmation might need different complementary techniques (GC- and LC-HRMS) using non-targeted approaches. The analyses are especially challenging because of the lack of scientific information on the metabolic fate of NPS; extensive metabolism in the body can lead to low or even negligible levels of the parent compound in urine, and consequently also in sewage. However, it is highly important to target the correct biomarker (parent compound or metabolite) to obtain reliable results.

Information on NPS use, together with data on the metabolic fate and health effects of NPS, should inform policy makers and stake holders (justice, public health authorities, and drug prevention workers) in order to perform health risk assessments and to eventually take targeted actions or legislative actions against NPS.